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Alteration of Behavioral Sex Differentiation by Exposure to Estrogenic Compounds during a Critical Neonatal Period: Effects of Zearalenone Methoxychlor, and Estradiol in Hamsters

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16. ABSTRACT

The present study was designed to determine if neonatal exposure to the estrogenic mycotoxin zearalenone or the weakly estrogenic pesticide methoxychlor could masculinize and/or defeminize the behavior of female hamsters. Neonatal hamsters were given a single s.c. injection of either zearalenone (1mg/pup), methoxychlor (1mg/pup), 17 βestradiol (E_2) (40 μg/pup) or the vehicle two days after birth. After puberty, behavioral estrous cyclicity was measured. The females were then ovariectomized, treated with the male hormone testosterone, and tested for their ability to mount a receptive female (a behavior not normally displayed by female hamsters).

Females treated neonatally with estradiol or zearalenone were masculinized but not defeminized, an effect consistent with perinatal exposure to low doses of sex hormones. Females in these two treatment groups displayed normal four day behavioral estrous cycles, but following ovariectomy and testosterone treatement they mounted a sexually receptive female at levels comparable to the males. Methoxychlor-treated females did not differ from contols.

The mounting behavior of similarly treated males was unaffected by any of the chemicals. However, males receiving estradiol treatment had smaller testes, seminal vesicles and cauda epididymides and 54% had epididymal cysts.

These results demonstrate that a single exposure to a weakly estrogenic chemical like zearalenone during a critical developmental period can cause the brain to differentiate in a manner inconsistent with the females genetic sex. This enables the female to respond to the activational influence of testosterone as an adult and readily mount a sexually receptive female.

The failure of methoxychlor to alter reproductive development in the current study may be due to an inability of the neonatal hamster to convert methoxychlor to estrogenic metabolites.

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Alteration of Behavioral Sex Differentiation by Exposure to Estrogenic Compounds during a Critical Neonatal Period: Effects of Zearalenone, Methoxychlor, and Estradiol in Hamsters[1]

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Alteration of Behavioral Sex Differentiation by Exposure to Estrogenic Compounds during a Critical Neonatal Period: Effects of Zearalenone, Methoxychlor, and Estradiol in Hamsters. GRAY, L. E., JR., FERRELL, J. M., AND OSTBY, J. S. (1985). *Toxicol. Appl. Pharmacol.* **80**, 127–136. The present study was designed to determine if neonatal exposure to the estrogenic mycotoxin zearalenone or the weakly estrogenic pesticide methoxychlor could masculinize and/or defeminize the behavior of female hamsters. Neonatal hamsters were given a single sc injection of either zearalenone (1 mg/pup), methoxychlor (1 mg/pup), 17 β-estradiol (E_2) (40 μg/pup), or the vehicle 2 days after birth. After puberty, behavioral estrous cyclicity was measured. The females were then ovariectomized, treated with the male hormone testosterone, and tested for their ability to mount a receptive female (a behavior not normally displayed by female hamsters). Females treated neonatally with estradiol or zearalenone were masculinized but not defeminized, an effect consistent with perinatal exposure to low doses of sex hormones. Females in these two treatment groups displayed normal 4-day behavioral estrous cycles, but following ovariectomy and testosterone treatment they mounted a sexually receptive female at a frequency comparable to the males. Methoxychlor-treated females did not differ from controls. The mounting behavior of similarly treated males was unaffected by any of the chemicals. However, males receiving estradiol treatment had smaller testes, seminal vesicles, and cauda epididymides and 57% had epididymal cysts. These results demonstrate that a single exposure to a weakly estrogenic chemical like zearalenone during a critical developmental period can cause the brain to differentiate in a manner inconsistent with the female's genetic sex. This enables the female to respond to the activational influence of testosterone as an adult and readily mount a sexually receptive female. The failure of methoxychlor to alter reproductive development in the current study may be due to an inability of the neonatal hamster to convert methoxychlor to estrogenic metabolites. © 1985 Academic Press, Inc.

While acute or subchronic exposure to environmental estrogens like diethylstilbestrol (DES), the pesticides chlordecone and methoxychlor, and the mycotoxin zearalenone can cause infertility in laboratory and domestic animals, these effects are usually reversible following cessation of treatment (Barnes *et al.*, 1983). Unlike these reversible reproductive effects, exposure to estrogens during critical periods of sexual differentiation can cause persistent alterations of reproductive development. For example, the gestational administration of the drug DES for treatment of threatened abortion in women was responsible for a vast array of reproductive problems in the offspring ranging from

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infertility to vaginal clear-cell adenocarcinoma (Herbst *et al.*, 1971). A number of investigators have mimicked the human effects of DES in rodents using either prenatal (Walker, 1983; McLachlan *et al.*, 1980) or neonatal (Ennis and Davies, 1982) injections of DES or estradiol. Neonatal DES administration is effective in rodents with a short gestation because the later events of sexual differentiation are perinatal rather than prenatal. Estrogenic treatments that result in pathological alterations following neonatal exposure in rats also cause specific areas of the brain of the female to differentiate like a male's (Gorski *et al.*, 1978; Dohler *et al.*, 1982). In conjunction with this central nervous system (CNS) alteration, these females are defeminized (i.e., they are incapable of displaying female sexual behavior) and infertile or become acyclic at an earlier age than untreated females. One area of the hypothalamus that is most obviously sexually dimorphic is the medial preoptic area, being about four times larger in the male (Gorski *et al.*, 1978). This sexual dimorphism develops when the testes of the neonatal male secrete testosterone which is in turn converted to estradiol in some areas of the CNS. Presently it is thought that estradiol is responsible for the sexual dimorphism of the preoptic area of the rat brain and for this reason injections of estradiol or DES cause enlargement of this area of the brain of the neonatal female as well (Dohler *et al.*, 1982).

Some other mammalian species, including primates, differ from rats as they are not defeminized by neonatal hormonal treatment. Hence, the significance of this phenomena to man has been questioned (Baum, 1979). Thus, in these other species, females are more readily masculinized in their behavioral patterns following neonatal hormonal treatment while they continue to cycle and breed normally. In this regard, the hamster is unique among rodents; females are more easily masculinized than defeminized. Neonatal treatment of the female hamster with 3 μg of testosterone propionate will masculinize females, enabling them to display male-like mounting behavior following testosterone treatment as adults, but defeminization occurs only at doses more than two orders of magnitude above this masculinizing dose (Whitsett and Vandenbergh, 1975). Neonatal estradiol treatment produces similar alterations of behavioral differentiation in the hamster (Whitsett *et al.*, 1978). We reported that the weakly estrogenic pesticide chlordecone alters sexual differentiation in a manner that is consistent with the results of studies using estrogen or testosterone (Gray, 1982). We found that a single injection of chlordecone during the critical period of behavioral sex differentiation in the hamster resulted in irreversible alterations of reproductive morphology and behavior in males and females respectively (Gray, 1982). Females treated neonatally with 500 or 1000 μg of chlordecone or 20 μg of estradiol benzoate were masculinized but not defeminized. These females had normal behavioral estrous cycles but displayed abnormal sexual behavior by mounting a receptive female. In addition to masculinization, higher doses of estradiol benzoate (100 μg) produced partial defeminization of female hamsters. Seventy-five percent of these females had abnormal behavioral estrous cycles, indicating that the release of gonadotrophins from the pituitary was tonic and more male like. These females retained the ability to display feminine sexual behavior being receptive more often than once every 4 days. The morphological development of the males was also affected although mounting behavior was not. Males exposed to the highest dose of chlordecone and estradiol had smaller testes and epididymides and 80% of the estrogen-treated males developed epididymal cysts.

The present investigation was designed to determine if zearalenone or methoxychlor administration during a critical period of sex differentiation would produce irreversible behavioral alterations in the female hamster or morphological lesions in the male. While these two chemicals both elicit weakly estro-

genic responses in the immature or adult rat and mouse uterine bioassay with about the same potency as chlordecone, they are about 1000-fold less estrogenic than estradiol itself (Kumagai and Shimizu, 1982; Bulger *et al.*, 1978). It is not certain that methoxychlor or zearalenone would alter reproductive differentiation in the neonatal hamster with the same efficacy as did chlordecone (Gray, 1982). This uncertainty exists because many biotransformation reactions have a low activity in the perinatal period (Klinger *et al.*, 1981), and the estrogenic effects of zearalenone and methoxychlor are at least partially dependent upon metabolic activation while chlordecone, in contrast, is not metabolized. Zearalanol, a metabolite of zearalenone, is five times as active as the parent compound (Ueno *et al.*, 1983) while methoxychlor seems to be completely devoid of estrogenicity unless metabolized (Bulger *et al.*, 1978).

METHODS

Timed-pregnant Golden Syrian hamsters were obtained from Engle Laboratories. Pregnant animals were individually housed in cages (23 × 15 × 45 cm) on pine shavings and maintained under conditions of constant temperature (22 to 24°C) and relative humidity (45 to 55%) with controlled lighting (14 hr light/10 hr dark, with lights out at 1100 EST). They were fed Wayne Lab Blox (Allied Mills) and tap water *ad libitum*. Laboratory grade zearalenone (100%; lot No. 023f4043) and methoxychlor (98%; lot No. 20f-5059) were purchased from Sigma Chemical Company.

In the present experiment zearalenone, methoxychlor, and estradiol (E_2), the free alcohol form, were given by sc injection in 20 μl of dimethylsulfoxide, analytical reagent grade (DMSO). All injections were administered when the pups were 2 days of age (Day 0 is the day of birth). The pups in six litters were injected with DMSO (injected controls), six litters were given 1 mg/pup of zearalenone, six litters were injected with methoxychlor (1 mg/pup), six litters were injected with 40 μg of E_2, and the fifth group of six litters was left undisturbed (uninjected controls). These dosages were selected from the literature which indicated that 1 mg of a weakly estrogenic compound was sufficient to alter behavioral sex differentiation. These reports indicate that the administration of 1 mg of zearalenone defeminizes neonatal female rats (Kumagai and Shimizu, 1982) and 1 mg of chlordecone masculinizes female hamsters (Gray, 1982). We elected to use 40 μg of E_2 because previous studies indicated that this would be an effective positive control for the present study (unpublished data). Our previous studies with hamsters and rats also demonstrated that the volume of DMSO used in the current experiment is without effect on growth, survival, or reproductive development (Gray, 1982). Pups were checked on Days 6, 7, 8, 9, 12, and 15, and the percentage of female pups with patent vaginas was determined. The pups were weaned at 21 days of age and housed in unisexual groups with littermates. The numbers of animals used in the present study are shown in Tables 1 through 4. The age at which the first behavioral estrus occurred, an index of puberty, was determined on Days 33 through 41 and behavioral estrous cyclicity was measured over one 4-day cycle starting at Day 60. Behavioral estrus was determined by placing the treated female in the home cage of a sexually active male and recording whether she displayed the posture of lordosis.

At 150 days of age the females were ovariectomized and a 25-mg pellet of testosterone propionate was implanted beneath the skin. Forty-five days later, Day 195, females were tested for the ability to mount a sexually receptive stimulus female hamster. At the onset of the dark period each experimental female was placed in the cage with a sexually receptive female. The number of head/side and rear mounts with pelvic thrusts displayed by the experimental female in a 3-min period following the first mount were counted. The experimental female was given 300 sec to mount the stimulus female or the test was terminated.

The males were paired with sexually receptive females on Day 225 and again at Day 280 and the mounting behavior was scored as in the experimental females. At 320 days of age the males were necropsied and body, testis, seminal vesicle, the tail of the epididymis, liver, kidney, adrenals, and Harderian glands were weighed and the flank gland diameter was measured. In addition the reproductive tracts were examined for lesions because estrogenic substances like estradiol and DES cause epididymal cysts when administered during the neonatal period as in the present study.

Experimental design. In the present experiment we elected to dose all pups within a litter with the same dose, treatment being a "between litter" effect, rather than using a "within litter" design in which each of the pups in a litter would receive a different treatment. This decision was based on previous analyses which indicated that litter effects on the behavior measured in the present study are undetectable (Gray, 1982) and our concern that the control pups might be exposed inadvertently to zearalenone or methoxychlor in the milk. Lactating dams routinely ingest their pups urine and fecal material in order to keep the nest area clean and they also cannibalize dead or moribund pups, including those dying as a result of treatment. In an earlier study we observed tremors in dams that cannibalized some of

their pups following chlordecone treatment of the neonates on Day 2 suggesting significant maternal exposure. Similarly, Gorski *et al.* (1977) reported that 100 μg of DES injected into neonatal rats on Day 4 actually sterilized noninjected littermates. Therefore, although a within-litter design is statistically preferable because potential litter effects are not confounded with treatment effects, it would be unacceptable for the present study and for many developmental toxicology studies.

Statistical methods. The parametric data in this study were analyzed by the General Linear Models (Proc GLM) available on the 1982 edition of the Statistical Analysis System (SAS User's Guide: 1982 edition). The percentage data, including the percentage of females with vaginal opening and the percentage mounting were analyzed by computing a normal deviate z, derived from the normal approximation to the binomial (Snedecor and Cochran, 1971). The z and the χ^2 test results are identical.

RESULTS

In the present study none of the neonatal treatments affected the subsequent growth or viability of the pups. In addition, DMSO injection did not affect any of the reproductive parameters measured in this experiment, i.e., they were all similar to the untreated control animals.

Estradiol accelerated vaginal opening as compared to all the other treatment groups, with 58% of the estrogen-treated females being open on Day 6 as compared to 0% in all the other groups (Table 1). By 7 days of age zearalenone had also significantly accelerated vaginal opening, with 17% open versus 0% for the controls and methoxychlor groups. The age at first behavioral estrus did not differ between any of the groups, occurring around 36 days of age and all of the females had normal behavioral estrous cycles when tested at 60 to 64 days of age (Table 2). These results indicate that zearalenone had a weakly estrogenic effect on the reproductive tract of the neonatal female, but neither zearalenone nor the dose of estradiol (free alcohol form) used in the current study were sufficient to defeminize the young females. However, it is possible that their cycles would have been abnormal had they been tested at an older age (Gorski *et al.*, 1977). While the zearalenone and estradiol-treated females were not defeminized by neonatal treatment they were clearly masculinized. Sixty-seven percent of the zearalenone-treated and 80% of the estradiol-treated females mounted a sexually receptive stimulus female as compared to 1 of the 30 control females (Table 2). The levels of mounting seen in these two

TABLE 1

THE EFFECTS OF NEONATAL ESTRADIOL, ZEARALENONE, OR METHOXYCHLOR EXPOSURE ON THE REPRODUCTIVE DEVELOPMENT OF THE FEMALE HAMSTER

	Neonatal treatment				
	Vehicle control	Untreated control	Methoxychlor	Zearalenone	Estradiol
Number observed	19	26	27	24	19
Day					
6	0	0	0	0	58[a]
7	0	0	0	17[a]	68[a]
8	21	8	18	21	63[a]
9	63	50	55	58	63
12	100	94	96	96	95
15	100	100	93	92	44[a]

Note. These data are the percentages of females with vaginal opening on Days six through 15.
[a] p less than 0.01 different from control by a z statistic.

TABLE 2

THE EFFECTS OF NEONATAL ESTRADIOL, ZEARALENONE, OR METHOXYCHLOR EXPOSURE ON THE REPRODUCTIVE BEHAVIOR OF THE FEMALE HAMSTER

	Vehicle control	Untreated control	Methoxychlor	Zearalenone	Estradiol
Age at first behavioral estrus	35.4 ± 0.5[a]	36.7 ± 0.6	33.1 ± 1.3	34.5 ± 0.3	36.o ± 0.6
Number observed	14	21	24	22	16
Percentage of females with normal behavioral estrous cyclicity	100%	100	100	100	100
Mounting behavior					
% Mounting	0%	6	0	66.7	80
Number mounts	0	0.8 ± 0.6	0	4.8 ± 1.3[b]	5.5 ± 1.2[b]
Number observed	13	17	16	15	15

Note. Behavioral estrus was observed in intact females who were subsequently ovariectomized, treated with testosterone, and then tested for their ability to mount a sexually receptive female.

[a] Values are $\bar{x} \pm$ SE.

[b] p less than 0.01 different from control by a t test.

groups was similar to the mounting behavior levels seen in the males (Table 3). Unlike zearalenone, methoxychlor treatment did not masculinize the treated females.

Neither zearalenone nor methoxychlor affected the reproductive morphology of the males in the present study (Table 4). The estrogen-treated males had smaller testes,

TABLE 3

THE EFFECTS OF NEONATAL ESTRADIOL, ZEARALENONE, OR METHOXYCHLOR ADMINISTRATION ON THE REPRODUCTIVE BEHAVIOR OF THE MALE HAMSTER

	Neonatal treatment				
	Vehicle control	Untreated control	Methoxychlor	Zearlenone	Estradiol
Trial 1 mounts					
Head/side	0.17 ± 0.17[a]	0.70 ± 0.40	0.73 ± 0.37	0.25 ± 0.25	0.83 ± 0.24
Rear	2.33 ± 0.83	0.80 ± 0.38	0.93 ± 0.39	0.88 ± 0.39	0.25 ± 0.17
Intro	8.00 ± 2.0	6.00 ± 1.3	5.87 ± 1.5	3.75 ± 1.3	4.08 ± 1.2
Trial 2 mounts					
Head/side	0.40 ± 0.40	0.90 ± 0.38	0.73 ± 0.41	0.47 ± 0.21	0.54 ± 0.27
Rear	1.40 ± 0.67	0.80 ± 0.13	0.60 ± 0.25	0.93 ± 0.35	1.23 ± 0.39
Intro	4.00 ± 2.07	7.80 ± 1.3	4.80 ± 1.3	3.00 ± 1.2	5.54 ± 1.3
Total mounts: both trials	15.4 ± 3.6	17.0 ± 2.5	14.6 ± 2.1	9.5 ± 2.1	12.2 ± 2.3
Number observed	5	10	15	15	12

Note. The data are for head and side mounts, rear mounts without intromission, and rear mounts with intromission.

[a] Values are $\bar{x} \pm$ SE.

TABLE 4

THE EFFECTS OF NEONATAL ESTRADIOL, ZEARALENONE, OR METHOXYCHLOR ADMINISTRATION ON ORGAN WEIGHTS OF MALE HAMSTERS AT 320 DAYS OF AGE

	Vehicle control	Untreated control	Methoxychlor	Zearalenone	Estradiol
			Necropsy Data		
Organ weights					
Number observed	6	10	16	17	14
Body (g)	140 ± 4[a]	141 ± 5	136 ± 4	140 ± 6	127 ± 7
Liver (g)	6.6 ± 0.6	5.7 ± 0.3	5.6 ± 0.3	5.7 ± 0.3	5.5 ± 0.3
Kidney (mg)	539 ± 13	534 ± 19	504 ± 22	527 ± 16	531 ± 27
Adrenals (mg)	30 ± 1.5	26 ± 1.2	25 ± 0.9	25 ± 1.1	30 ± 3.2
Harderian gland (mg)	241 ± 9	249 ± 3	243 ± 6	241 ± 5	227 ± 6
Testis (g)	1.93 ± 0.04	1.87 ± 0.07	1.81 ± 0.10	1.75 ± 0.12	1.40 ± 0.19[b]
Seminal vesicle (g)	1.76 ± 0.13	1.89 ± 0.07	1.81 ± 0.07	1.79 ± 0.13	1.28 ± 0.18[b]
Cauda epididymis (mg)	437 ± 14	424 ± 14	396 ± 20	401 ± 12	353 ± 26[b]
Percentage of males with epididymal cysts	0	0	0	0	57[b]
Flank gland diameter (mm)	6.53 ± 0.41	5.72 ± 0.12	6.01 ± 0.10	6.03 ± 0.15	4.65 ± 0.53[b]

[a] Values are $\bar{x}$ ± SE.
[b] p less than 0.01 different from the controls.

seminal vesicles, flank glands, and cauda epididymides and 57% of them had epididymal cysts. The mounting behavior of the males was unaffected by any treatment even though many of the estrogen-treated males had epididymal cysts at necropsy.

DISCUSSION

In the present study a single injection of the mycotoxin zearalenone or estradiol on Day 2 altered the normal pattern of sexual differentiation. When exposed to testosterone as an adult the zearalenone and E_2-treated females mounted a sexually receptive female, a behavior that is not displayed by the control females. Although masculinized, the zearalenone and estradiol-treated females were not defeminized. The age at first behavioral estrus (puberty) and behavioral estrous cyclicity were normal. This type of chemical alteration of sexually dimorphic behavioral development is possible because there are a number of critical developmental stages during which the mammalian embryo differentiates from an indifferent state with bisexual potential into a male or a female (Naftolin, 1981). Following genetic sex determination at fertilization the H-Y antigen in the male induces the indifferent gonad to become a testis, while the absence of this organizing factor results in the formation of an ovary (Gordon and Ruddle, 1981; Haseltine and Ohno, 1981). Subsequently, the fetal testis produces testosterone and other factors which cause the male tract to develop and the female tract to regress. The lack of these endocrine signals result in the persistence of a female tract and regression of the male tract (Wilson *et al.*, 1981). Similarly, the mammalian fetus also possesses bisexual behavioral potential. When present during a critical period androgen induces specific areas of the brain to differentiate into a male brain, whereas the lack of testicular hormones causes the brain

to differentiate as a female brain. In all mammalian species studied to date sexual differentiation of the brain is associated with permanent anatomical differences between males and females. In the rat, the species studied in the greatest detail, nuclei in the preoptic area is considerably larger in the male than the female. This difference appears in the first week of life (Jacobson *et al.*, 1980) and is independent of pubertal hormonal activation. The brain metabolizes testosterone to estradiol and the estrogen induces the differentiation process for some areas of the brain. Thus, DES, estradiol, and presumably chlordecone and zearalenone can induce this effect in the female because of their estrogenic effect on the CNS. The brain of the male and the masculinized female is then able to respond to the activational influence of testosterone as an adult (Whitsett *et al.*, 1978). This activation occurs at puberty in the male as the testes begin to secrete testosterone, while in the masculinized female behavioral activation occurs after the testosterone pellet is implanted. In the hamster, masculinization of the brain is much more easily attained by neonatal hormonal manipulations than is defeminization and the doses of the estrogenic chemicals used in the present study were not sufficient to cause defeminization. In an earlier study we found that chlordecone also masculinized, but did not defeminize the hamster, while 100 μg of estradiol benzoate partially altered both developmental events (Gray, 1982). We had anticipated that zearalenone would masculinize the female hamsters because it has estrogenic effects in both domestic and experimental animals. Swollen vulva, vaginal prolapse, and enlarged uterus were observed in the prepubertal gilt exposed to the compound (Mirocha *et al.*, 1977). In experimental animals the compound has a uterotropic effect, it induces vaginal cornification, inhibits ovulation and implantation, suppresses gonadotropic hormone release (Hidy *et al.*, 1977; Hobson *et al.*, 1977), and interacts with the estrogen receptor in uterine cytosol of rat and mouse (Greenman *et al.*, 1979; Katzenellenbogen *et al.*, 1979). In addition neonatal exposure to zearalenone defeminizes female rats causing persistent anovulatory estrus (Kumagai and Shimizu, 1982).

While zearalenone was effective in masculinizing the females this compound did not alter reproductive development of the males. The estrogen-treated males had smaller testes, seminal vesicles, cauda epididymides, and flank glands and 57% had epididymal cysts while the zearalenone-treated males were similar to control males. In an earlier study we found that males neonatally exposed to estrogen or chlordecone had smaller testes and epididymides as adults than the control males while estradiol treatment also caused epididymal cysts. A comparison of the results of these two studies indicates that zearalenone is a more potent estrogen than methoxychlor in the neonatal hamster but less so than chlordecone, tested previously (Table 5). The effect of chlordecone on vaginal opening was more robust than the slight acceleration induced by zearalenone in the present study and, of the two compounds, only chlordecone altered reproductive morphology in the male. In addition, the estradiol used in the present study was the free alcohol form and 40 μg of it was less potent than 20 or 100 μg of the esterified form of estradiol, estradiol benzoate, which we used in the chlordecone study (Gray, 1982). The difference in potency between the free and esterified forms of estradiol has been reported in all systems in which estrogenic effects have been studied.

Methoxychlor is also a weak estrogen but it failed to elicit any estrogenic effects in the neonatal hamster. While methoxychlor is clearly estrogenic in adult and immature rats even when administered as a single dose below that used in the present study (Bulger *et al.*, 1978), i.e., it stimulates uterine growth in mice and rats and dietary administration blocks ovulation in females and causes testicular atrophy in male rats (Hodge *et al.*, 1950), the estrogenicity of the purified compound has never been assessed in neonatal

TABLE 5

A COMPARISON OF THE ESTROGENIC EFFECTS NOTED IN THE PRESENT STUDY OF ZEARALENONE, METHOXYCHLOR, AND FREE ESTRADIOL WITH THOSE FROM AN EARLIER STUDY (GRAY, 1982) WHICH USED CHLORDECONE AND ESTERIFIED ESTRADIOL, ESTRADIOL BENZOATE

	Neonatal treatment				
	Methoxychlor (1 mg)	Zearalenone (1 mg)	Chlordecone (1 mg)	Estradiol (40 μg)	Estradiol benzoate (100 μg)
Effect					
Masculinization of females	No	Yes	Yes	Yes	Yes
Accelerated vaginal opening	No	Slight	Yes	Yes	Yes
Reduced testes weight in males	No	No	Yes	Yes	Yes
Reduced epididymal weight	No	No	Yes	Yes	Yes
Epididymal cysts	No	No	No	Yes	Yes
Partial defeminization of female behavior	No	No	No	No	Yes
Interference with normal masculinization in the male	No	No	No	No	No

Note. The variables are ranked in order of sensitivity to perturbation by neonatal estrogen exposure.

rats and mice, or in hamsters of any age. The difference between laboratory and technical grades of methoxychlor is significant. The purified compound is not active per se, but must be metabolized by the hepatic monooxygenase into active phenolic metabolites, while technical preparations contain contaminants which are largely responsible for the estrogenic activity of crude samples *in vitro* (Kupfer and Bulger, 1980). It has been reported that technical grade methoxychlor can alter sexual differentiation when fed to dams during gestation and lactation (Harris *et al.*, 1974) but in the current study a single neonatal injection of the purified material had no effect. It is possible that purified methoxychlor was ineffective because the neonatal hamster cannot metabolize this compound rapidly enough during the critical period to alter reproductive differentiation. In short-term gestation rodents like the hamster (gestation length = 16.5 days) the monooxygenases are absent or barely detectable until just before birth and their activities are low in the perinatal period (Klinger *et al.*, 1981). It is possible that the dose used in the present study would be potent enough to produce an effect in the neonatal rat, but not in the hamster. Repeated dosing, or higher doses of methoxychlor than the one used in the present study may be required to masculinize the female hamster. It is known that higher doses of estrogens are often required in the hamster to elicit the same effects seen in rats (Baranczuk and Greenwald, 1972). If methoxychlor is indeed ineffective in the neonatal rat and hamster after repeated dosing, the use of the neonatal rat to screen chemicals for estrogenic effects as recommended (USEPA-600/9-82-001, 1980) is in question.

While the results of our studies demonstrate the potential of weak environmental estrogens like chlordecone and zearalenone

to alter behavioral sex differentiation in the female hamster the extrapolation of such effects to the human is worthy of comment. As stated earlier we elected to use hamsters because they resemble primates, being easily masculinized by androgen treatment but not defeminized. Testosterone administration to pregnant rhesus monkeys between Days 40 and 100 of gestation did not defeminize the female offspring's behavior but puberty was delayed (29.2 months versus 36.8) and these females showed patterns of play behavior which were similar to those of males during the first 2 years of life and they increased their display of mounting before puberty (Resko, 1975). Aromatization of testosterone has been demonstrated in fetal monkey brain (Naftolin *et al.*, 1975) but no studies have evaluated role of estrogens in behavioral sex differentiation of this species. In man, the evidence accumulated to date suggests that human psychosexual differentiation is influenced by prenatal hormones, albeit to a limited degree. Best established are the effects of prenatal androgens on intense active outdoor play and physical energy expenditure (Ehrhardt and Meyer-Bahlburg, 1981). However, little information is available on the ability of testosterone metabolites or estrogenic substances like DES to regulate human behavioral sex differentitation. There is some evidence that the age at puberty is affected by testosterone exposure during a critical period but not by estrogens. The age of puberty (menarche) was not delayed in either DES-exposed women (Wu *et al.*, 1980) or in estrogen-treated female hamsters in our studies although puberty is delayed by testosterone exposure in hamsters, rhesus monkeys, and man. In summary, while it is clear that behavioral masculinization occurs in all mammalian species, including man, the degree to which this process is dependent upon the aromatization of testosterone to estradiol has yet to be determined, and hence the potential for compounds like DES, zearalenone, and chlordecone to elicit this effect in human females *in utero* is presently unknown.

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